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REMARKS

Claims 12 and 23 have been amended to indicate that the fermentation medium contains water. The fermentation medium of the present invention is the standard type of fermentation medium which has met with great success in the production of a great variety of products by the biodegradation process.

The present invention is based on the unexpected discovery that if a hydrophobic substrate or co-substrate is utilized in the fermentation process and the hydrophobic substrate or co-substrate is introduced into the fermentation medium in the form of a oil in water microemulsion, the amount of mechanical energy and stress required to insure that an adequate amount of oxygen is dissolved in the fermentation medium can be substantially reduced. Applicants respectfully submit that the use of an oil in water microemulsion for introducing a hydrophobic substrate or co-substrate into a fermentation medium and the reduction in the energy input required to adequately oxygenate the fermentation medium was unknown and unexpected in the prior art. Applicants therefore respectfully request favorable consideration and allowance of the application.

Claims 12-22 stand rejected under 35 USC 103(a) as unpatentable over Tellier et al. (US 4,401,762) and Inlow et al. (US 5,372,943) in view of Kopp-Holtwiesche (DE 3738812) and Forster et al. (WO 95/11660). Applicants respectfully submit that Tellier et al., Inlow et al., Kopp-Holtwiesche and Forster et al. whether considered alone or in combination neither teach nor suggest the present invention.

Tellier et al. is directed to a process for culturing of microorganisms using a

microemulsion. Tellier et al. differs from the present invention in that the medium required in the Tellier et al. invention is a hydrophobic medium and the process introduces the water soluble micro nutrients into the hydrophobic material in the form of a water-in-oil microemulsion. It is critical in the Tellier et al. process that the microemulsion be a water-in-oil microemulsion since when introduced into the hydrophobic medium, the continuous hydrophobic or oil phase dissolves in the hydrophobic medium (dilutions of the microemulsion with the hydrophobic medium). This permits the small particles of the aqueous phase containing the water soluble micro nutrients to disperse in the hydrophobic medium. The process provides for intimate contact between the aqueous particles containing the water soluble nutrients with the hydrophobic medium. There is neither teaching nor suggestion that the water-in-oil emulsion would reduce the amount of mechanical energy required to provide an adequate supply of oxygen to the fermenting medium. This is not important in the Tellier et al. process since the process is designed to handle degradation of oil slicks on open water or oil contaminated grounds, the oxygen required for the degradation of the hydrophobic components is provided by natural phenomena such as wind and the like.

The Tellier et al. invention is based on the use of a water-in-oil microemulsion which is necessary and critical to the process. Applicants therefore respectfully submit that Tellier et al. would neither teach nor suggest to one skilled in the art to utilize an oil-in-water microemulsion in a fermentation process.

Inlow et al. is directed to utilizing lipid microemulsions to provide essential lipids to a

fermentation process. The lipids are essential growth components in serum and albumin free culture media (see Fig. 1). The essential lipids are provided to the culture medium in the form of a microemulsion of the oil-in-water type in relatively small amounts and apparently do not act as a substrate or a co-substrate in the fermentation process. The microemulsion disclosed in Inlow et al. contains relatively minor amounts of the lipids dissolved in an organic solvent which is emulsified to form the microemulsion. The lipids are defined as any of a heterogeneous group of fats and fatlike substances characterized being water-insoluble and being extractable by non-polar (or fat) solvents such as alcohol, ether, chloroform or benzene. The lipids include unsaturated fatty acids, saturated fatty acids, sterols, lipid soluble vitamins and phospholipids and their esters. The unsaturated fatty acids include linoleic acid, linolenic acid, oleic acid, arachidonic acid and their glycerides and esters. Preferred fatty acids include fatty acid esters, preferably polyunsaturated fatty acid esters and still more preferably, mixtures of polyunsaturated fatty acid methyl esters. The lipids are supplied in concentrations appropriate for the particular cell line being cultured, when the concentration is non-toxic to cells it is non-inhibitory to cell growth. At col. 5, lines 10-21, Inlow et al. teaches polyunsaturated fatty acid methyl esters, such as, fish liver oil, preferably cod liver oil, is preferably present in the media for insect cells at a concentration of 1 mg/l to about 50 mg/l, preferably from about 5 mg/l to about 15 mg/l, and most preferably about 10 mg/l. Media for mammalian cells contains less lipids than for insect cells. The medium can contain from about 2 mg/l to about 7 mg/l of cholesterol, from about 0.5 mg/l to about 4 mg/l of alpha tocopherol.

As can be seen from the teachings of Inlow et al., the amount of the growth promoting lipid supplement to the serum free and albumin free medium is relatively low and remains in the media for at least about 200 hours.

In contrast to the teachings of Inlow et al., the present invention provides fatty acid esters to the fermentation medium in the form of an oil-in-water microemulsion as a substrate or a co-substrate. By its definition, the substrate or co-substrate is oxidized during the fermentation process. The substrate is oxidized to provide the product of the fermentation and the co-substrate is oxidized to provide energy for the process. Applicants submit that one skilled in the art would not provide a substrate or co-substrate in the fermentation medium at a level of 10 mg/l and expect any kind of a reasonable production of the desired product.

Applicants invite the Examiner's attention to the article "Production of Erythromycin and Triketide Lactone by *Saccharopolyszpora erythraea* in Rapeseed Oil at Two Different Scales", Noushin Mirjalili, et al.

The article "Methyl Oleate-Based Fermentation Medium for Cephalosporin C Production", Chao-Han et al., Smith Kline & French Laboratories, Department of Natural Products Pharmacology, Philadelphia, Pennsylvania, presents examples for the production of cephalosporin C utilizing fermentation mediums containing methyl esters and triglycerides as a partial carbon source during the fermentation. When the triglycerides or methyl oleate were utilized as a partial carbon source, the esters were continuously added to the fermentation medium during the process. At any rate, the amount of the methyl

oleate in the ester when it was a minor portion of the carbon source was at 0.6% by weight and was at 2% by weight when it was a major portion of the carbon source with continuous introduction to the reaction and fermentation medium during the process. Both references are presently of record in the application.

From the two references cited, Applicants submit that the introduction of a triglyceride or a fatty acid ester as a substrate or a co-substrate into a fermentation process involves amounts far above the levels disclosed in Inlow et al. In addition, since Inlow et al. clearly discloses at Fig. 1 that the small amount of the cod liver oil (fatty acid ester) remain in the fermentation medium for at least about 160 hours, the fatty acid ester or triglyceride would not be considered as a substrate or a co-substrate which are considered as reactants in the fermentation. The components in the microemulsion of Inlow et al. clearly relates to the proliferation of cell growth by means other than the oxidation of the diglyceride or fatty acid ester included in the microemulsion. Applicants respectfully submit that the Examiner's assertion that the microemulsion disclosed in Inlow et al. would be considered as a substrate or a co-substrate in the fermentation process is untenable in view of the prior art which discloses that the substrates and co-substrates are present in the range of about at least 6 g/l in contrast to the maximum of 50 mg/l useful in the Inlow et al. process. Clearly, Tellier et al. and Inlow et al. bear no relation to the present invention. Applicants submit that to be useful as a substrate or a co-substrate, the fatty acid esters or triglycerides useful in the practice of the present invention must be added to the fermentation medium in substantial amounts to provide the substrate which is transformed

or to provide the co-substrate which provides the energy for promoting cell growth and activity.

The deficiencies in the combination of Tellier et al. with Inlow et al. are not cured by combination with Kopp-Holtwiesche. Kopp-Holtwiesche discloses the use of methyl laurate as a substrate for forming alpha, omega-dicarboxylic acid in a fermentation process. However, there is neither teaching nor suggestion that there would be any advantage for introducing the methyl laurate into the fermentation medium in the form of an oil-in-water microemulsion. Applicants respectfully submit that it has been well known for many years that fatty acid esters including the triglycerides can be utilized as substrates and co-substrates in fermentation processes. However, there is neither teaching nor suggestion that an oil-in-water microemulsion of the fatty acid ester or fatty acid triglyceride would have any advantageous effects on the fermentation process.

The deficiencies in the combination of Tellier et al. with Inlow et al. and Kopp-Holtwiesche are not cured by combination with Forster et al. Forster et al. is directed to the preparation of PIT oil-in-water emulsions. Again, PIT oil-in-water emulsions were well known before the present invention, however, to Applicants' knowledge they have never been applied to introducing fatty acid esters or triglycerides into a fermentation medium as a substrate or a co-substrate.

The Examiner states that cosmetic formulations such as disclosed in Forster et al. are in themselves fermentation medium. Applicants respectfully request that the Examiner reconsider the understanding of a fermentation medium. As disclosed in the present

application, the fermentation medium is a composition containing a nutrients energy sources and micro nutrients required to promote the growth of certain microorganisms. The fermentation mediums are generally sterilized and promote the growth of a particular microorganism. In addition, the PIT emulsion is only a small part of the fermentation medium. That is, the microemulsion useful in the practice of the present invention is a microemulsion of the fatty acid ester and/or a fatty acid triglyceride with emulsifiers in water. The microemulsion useful in the practice of the present invention does not contain other components which are not tested to be certain that they do not affect, in a negative way, the growth of the desired microorganisms in the fermentation medium. The microemulsion of the present invention is neither similar to nor suggested by Forster et al. which discloses a complete cosmetic formulated composition in the form of a PIT microemulsion. Applicants submit that the microemulsion disclosed in Forster et al. bears little relation to the microemulsion utilized in the practice of the present invention.

The Examiner appears to be reading the prior art in a vacuum divorced from any real world reality to arrive at the conclusion that the PIT microemulsion disclosed in Forster et al. is equivalent to the fermentation medium useful in the practice of the present invention.

According to the Examiner's analysis of the prior art, any organic material which contained water should be considered as a fermentation medium. Applicants submit that it is well known in the art since the time of Pasteur that microorganism growth precedes from prior microorganisms which are ubiquitous in the environment and when a particular

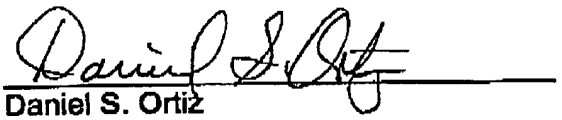
microorganism comes in contact with an organic substrate which contains water, there is a probability that the microorganism can proliferate if it is amenable to use of the substrate for growth. However, the fermentation medium which is useful in the practice of the present invention, is generally sterilized to be certain that unwanted microorganisms are not present then inoculated with a particular microorganism to produce a particular product of interest to the operator of the process. Applicants respectfully submit that this is far different from the unintended spoilage of organic materials due to the unintended action of ubiquitous microorganisms. As noted by the Examiner, the PIT microemulsions such as disclosed in Forster et al. would generally contain microorganism growth inhibiting materials to prevent the proliferation of unintentionally induced microorganisms.

In addition, as stated above, the PIT microemulsion disclosed in Forster et al. is a complete composition and would be equivalent to the complete fermentation medium prepared in the present invention. However, the microemulsion useful in the practice of the present invention is only a small portion of the total fermentation medium prepared for cultivation of a particular microorganism to produce a particular desired product. Applicants therefore respectfully submit that comparison of the PIT microemulsion prepared in Forster et al. to the microemulsion useful in the practice of the present invention, which is only a small portion of the fermentation medium, is untenable and is divorced from any interpretation of the claims in the present application or any reality in the world of fermentation processes to produce useful products. The Examiner appears to be comparing the entire fermentation medium which is not a PIT microemulsion with the

microemulsion product disclosed in Forster et al. Applicants respectfully submit that this is not a valid comparison and would neither teach nor suggest the present invention.

In view of the amendments entered in the claims and the above discussion, Applicants respectfully submit that the application is in condition for allowance and favorable consideration is requested.

Respectfully submitted,



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